



Research

***A streamlined LC-MS/MS platform for
the screening and confirmation of 152 veterinary drug residues
in a broad range of food raw materials, processed ingredients
and finished products***



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132nd AOAC Annual Meeting & Exposition

Toronto, Canada, August 26-29, 2018

Veterinary Drugs

Definition

Any substance applied or administrated to any food-producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behaviour.

Use

To treat existing illness, to prevent future disease, and promote growth.

Pharmacological actions

ANTI biotics
inflammatories
parasitics

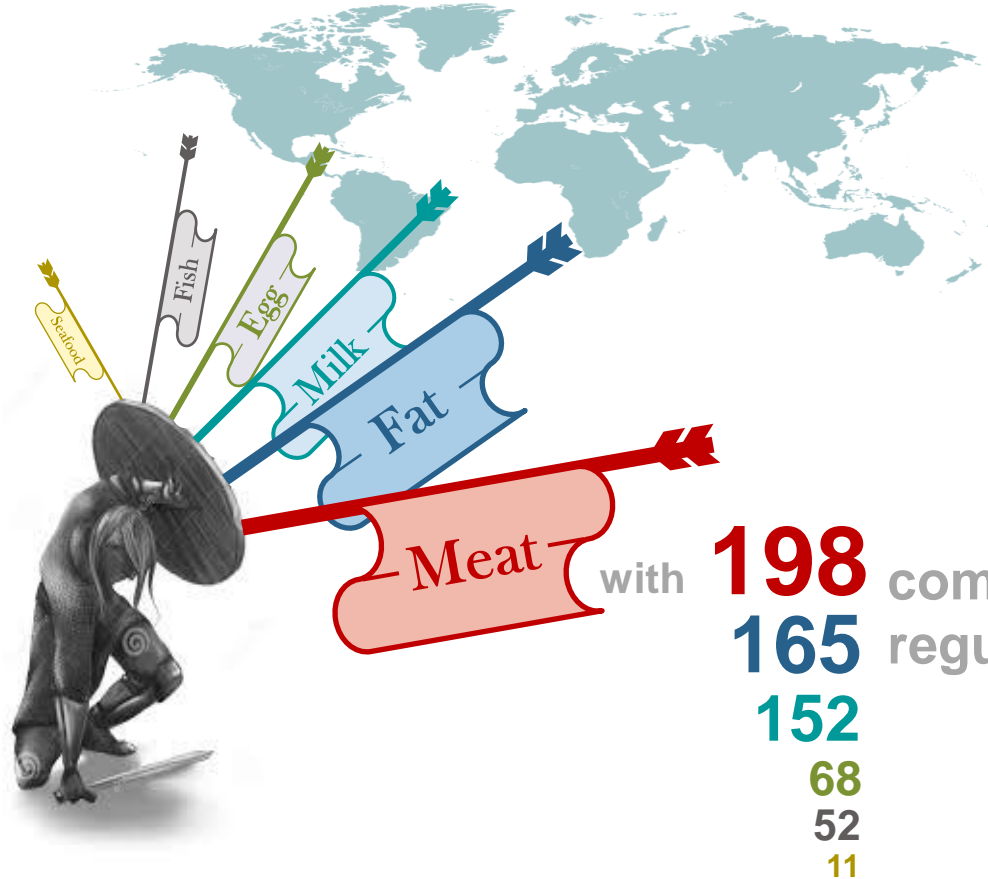
Tranquiliz
Painkill
Growth-promot **ERS**



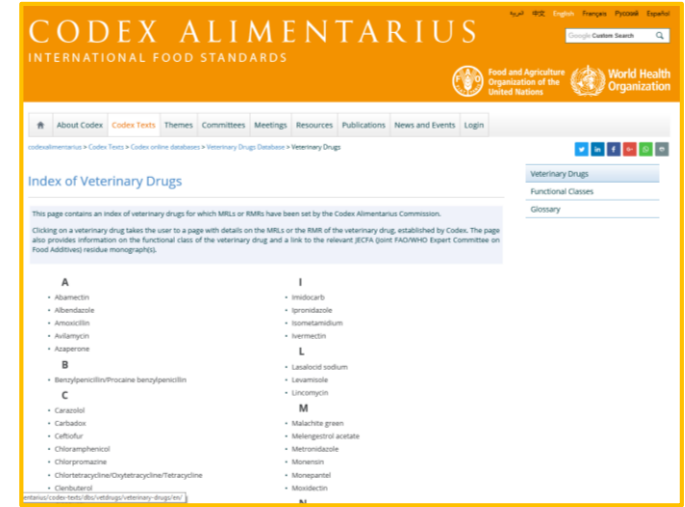
Research

Regulatory framework

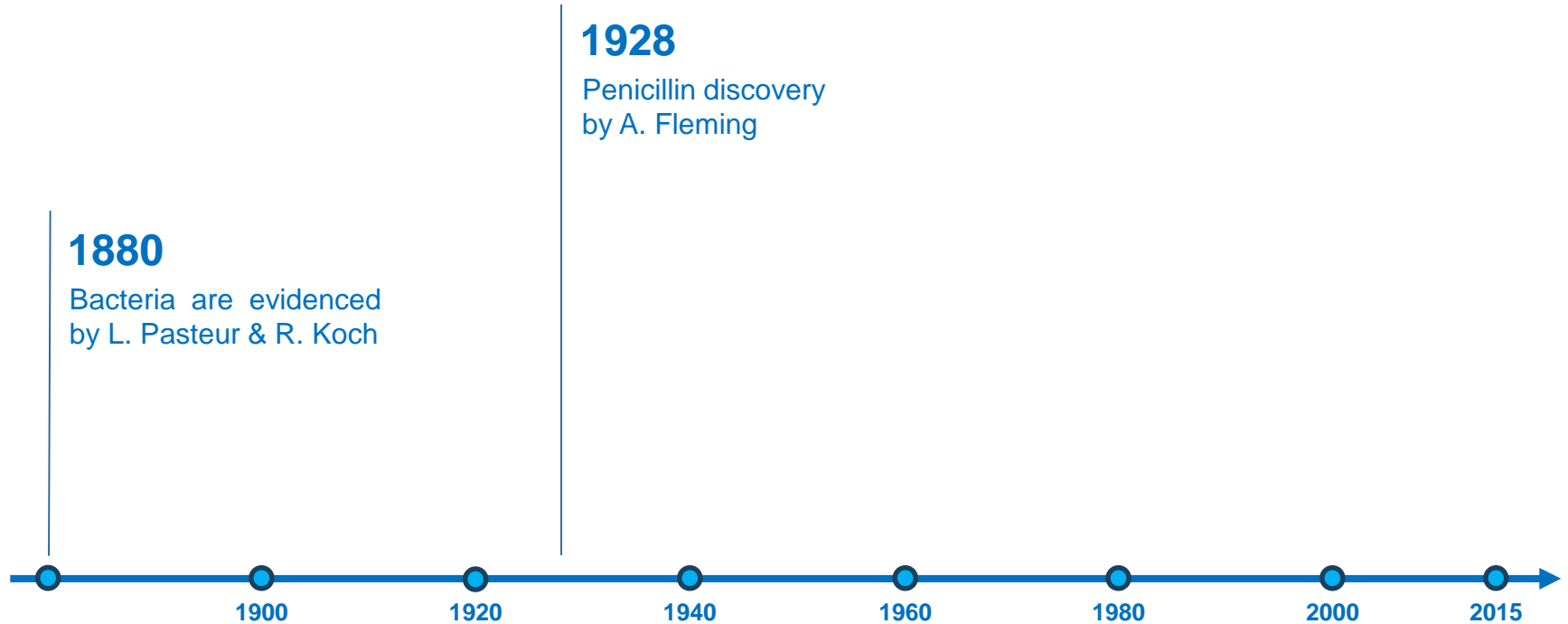
Source: EU, USA, Canada, China, Codex Alimentarius



with **198** compounds
165 regulated
152
68
52
11



Antibiotics at a glance

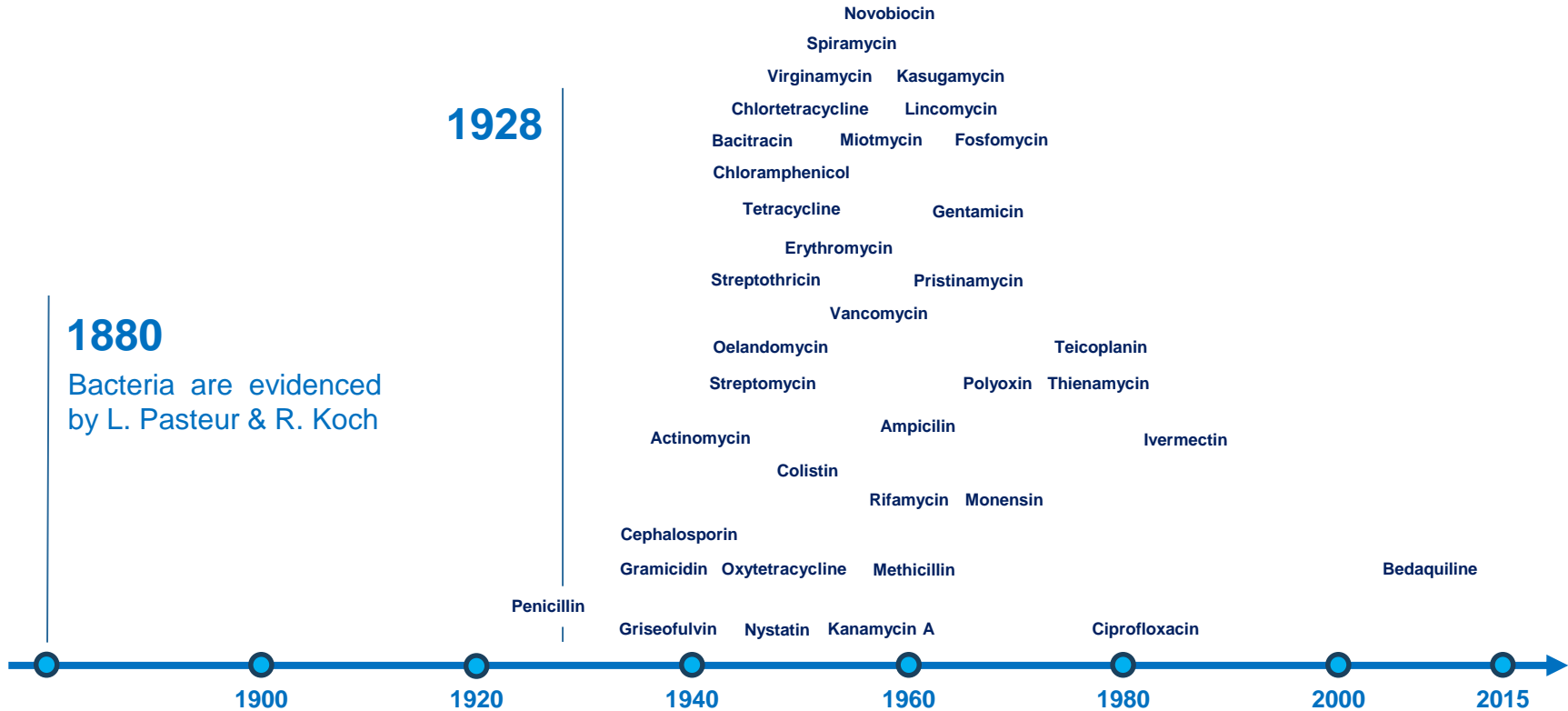


Source: Le Monde, 2017, La santé & la médecine.



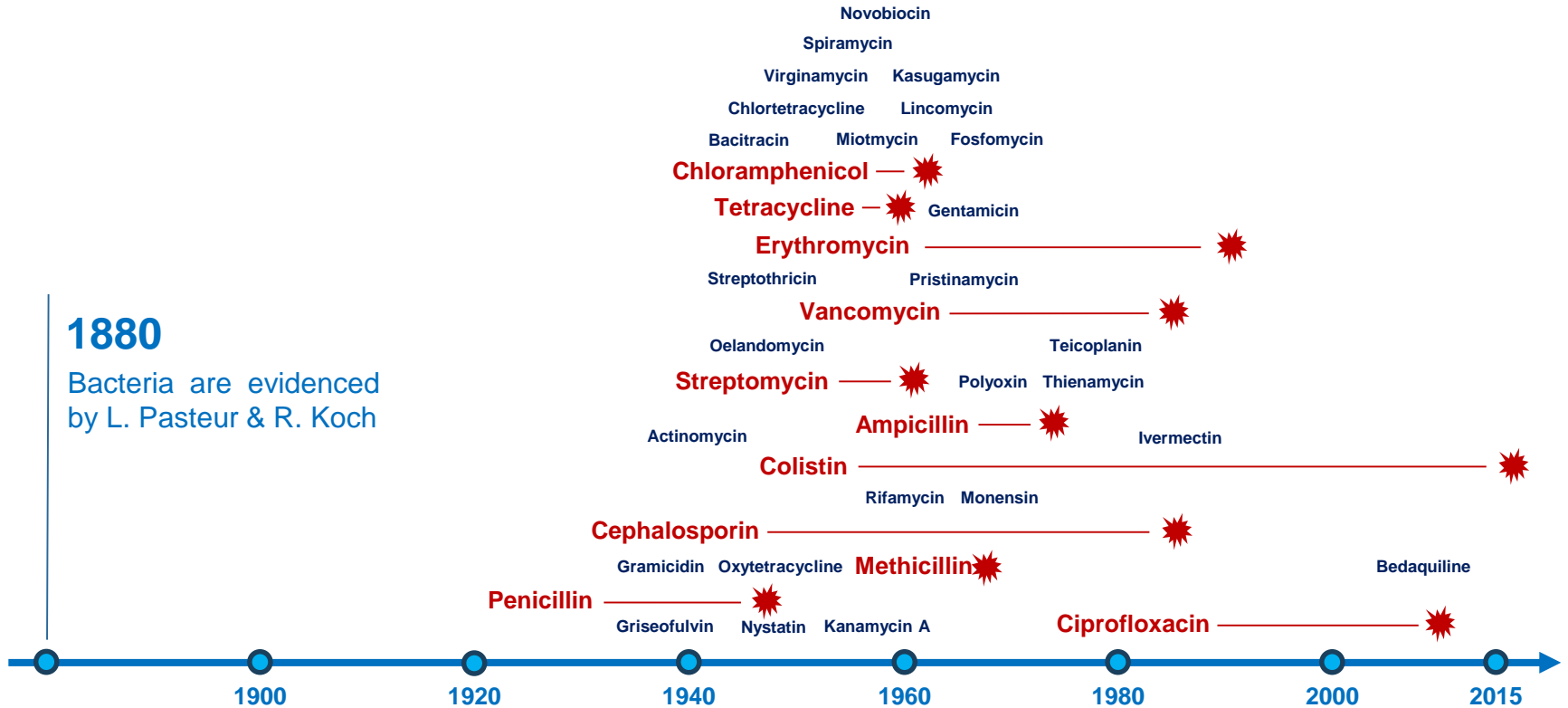
Research

Antibiotics at a glance



Antibiotics at a glance

 First antimicrobial resistance evidenced



1880

Bacteria are evidenced by L. Pasteur & R. Koch

Food materials

- **Milk**
- **Meat**
- **Fish/seafood**
- **Egg**

Food materials



Milk

Raw Whole milk powder Fat-filled milk powder Skim milk powder Whey protein powder Lactose Casein



Meat

Fresh Frozen Powder



Fish/seafood

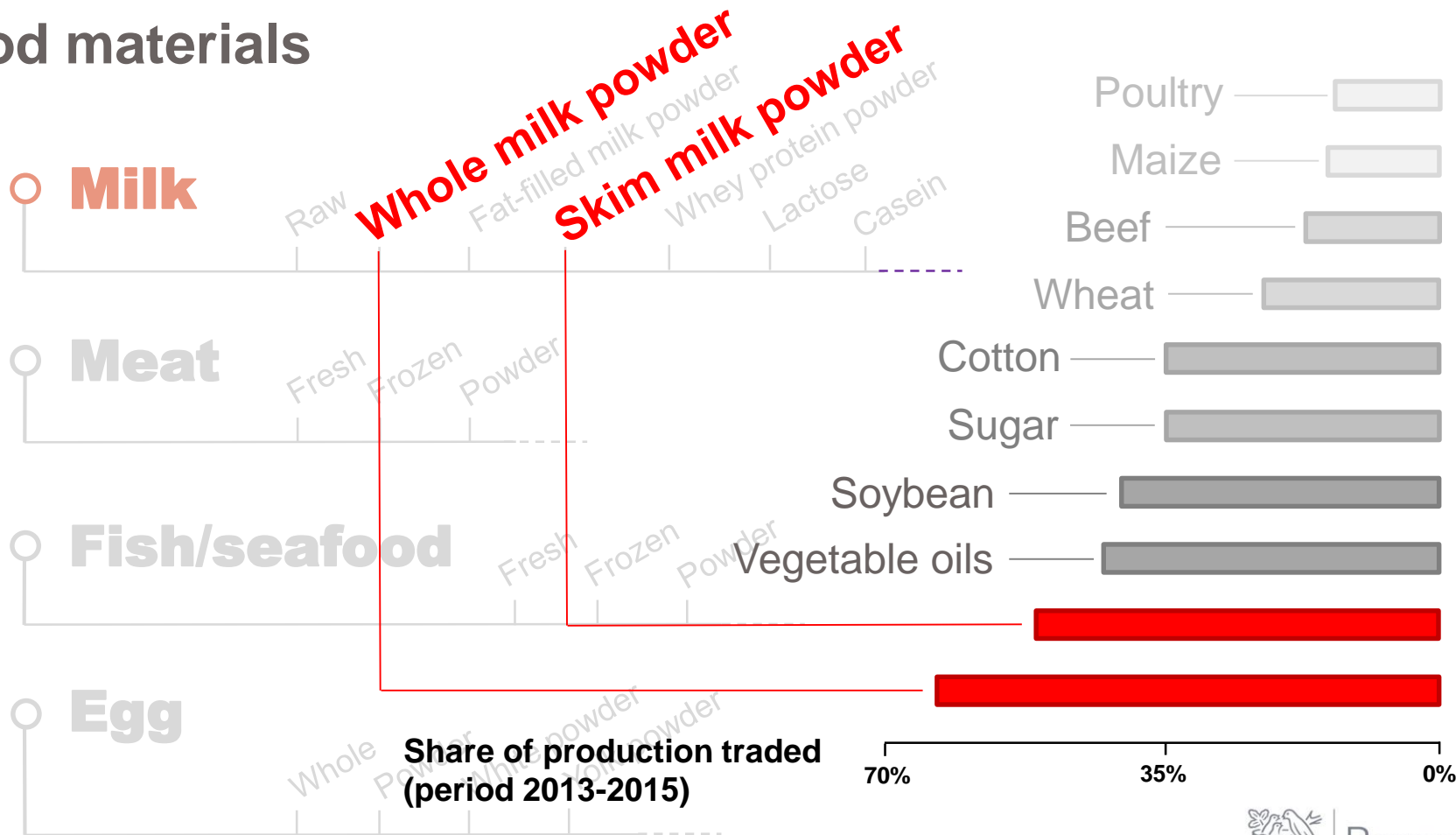
Fresh Frozen Powder



Egg

Whole Powder White powder Yolk powder

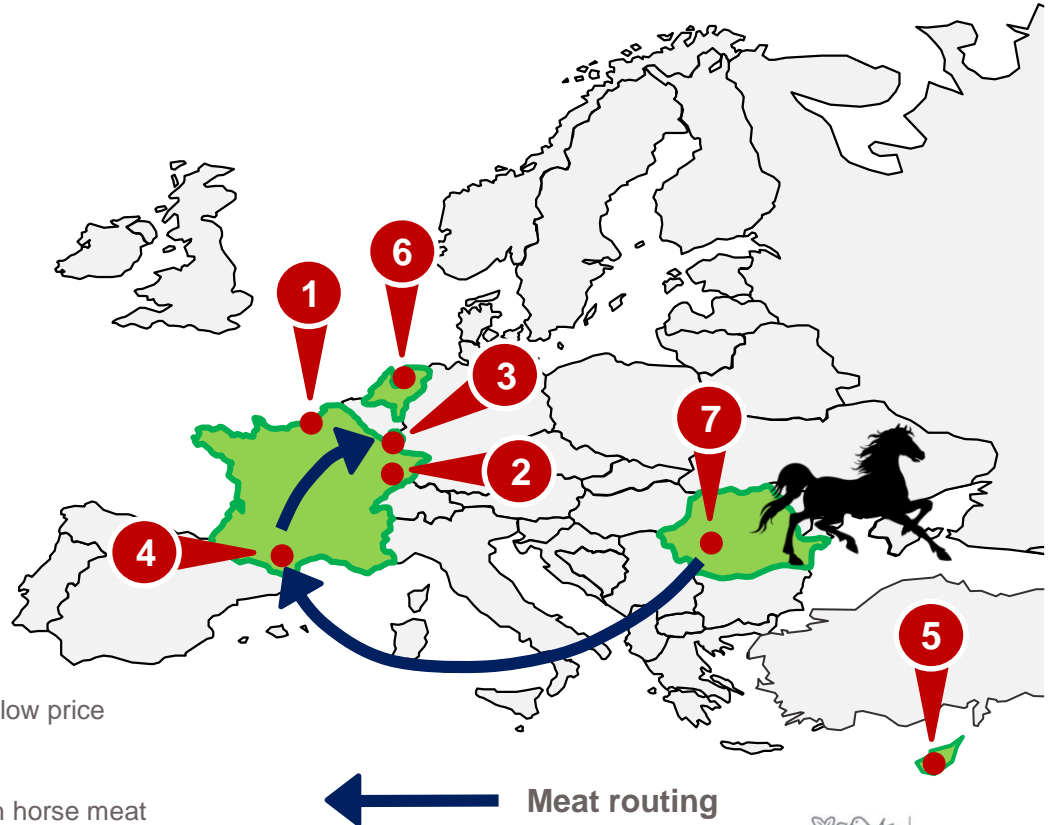
Food materials



Source: OECD/FAO, 2016, OECD-FAO Agricultural Outlook

Complexity of the supply chain

- 1 Boulogne-sur-Mer (France)**
Findus orders beef-meat lasagnes
- 2 Metz (France)**
Comigel requires *Tavola* to manufacture the product
- 3 Capellen (Luxemburg)**
Tavola orders fresh meat to *Spanghero*
- 4 Castelnaudary (France)**
Spanghero requires a trader to purchase
- 5 Cyprus**
The trader subcontracts the order to another trader in the Netherlands
- 6 The Netherlands**
The Dutch trader orders the fresh meat in Romania at low price
- 7 Romania**
A Romanian slaughterhouse provides *Spanghero* with horse meat



Non-compliant sample rates

MILK DRUG RESIDUE SAMPLING SURVEY

1.15 %
n = 953 samples

2015

2014 on the results from the monitoring of primary medicinal product residues and other substances in live animals and animal products

European Food Safety Authority

0.37 %
n = 736'907 samples

Abstract
 The report summarizes the monitoring data collected in 2014 on the presence of residues of pharmaceutical products and certain substances in live animals and animal products in the European Union. A total of 736'907 samples were reported to the European Commission by the 28 EU Member States. They consisted of 453,522 targeted samples and 14,097 support samples reported under Council Directive 96/23/EC and of 4,156 samples collected at import and 29,142 samples collected in the framework of programmes developed under the national legislation. The majority of Member States fulfilled the minimum requirements for sampling frequency set down in Council Directive 96/23/EC and in Commission Decision 97/747/EC. The percentage of non-compliant targeted samples (0.37%) was slightly higher compared to the previous 7 years (0.25% in 2013). The overall results, however, do not indicate any trend for an increase or decrease. Overall, the number of non-compliant samples for prohibited substances was reported in 2014, compared to the previous 7 years. In the other substance groups, there were no notable variations over the 8 year period. This evidence should be regarded as having a certain degree of uncertainty, as it is based on partially aggregated data and the sampling plans and the spectrum of substances analysed are not necessarily the same every year.

© European Food Safety Authority, 2016
 Key words: veterinary medicinal products, residue monitoring, Directive 96/23/EC, food safety

Requester: European Commission
 Question number: 9794-Q-12-015-0001
 Correspondence: b10ccant@efsa.europa.eu

2016

Determination of five tetracyclines and their epimers by LC-MS/MS based on a liquid-liquid extraction with low temperature partitioning

0.81 %
n = 2'600 samples

Abstract
 Tetracycline (TET) and its epimers are common antibiotics used in the treatment of a wide range of bacterial infections. The presence of these antibiotics in animal products is a concern for public health. This study describes the development of a sensitive and specific LC-MS/MS method for the determination of five tetracyclines and their epimers in animal products. The method involves liquid-liquid extraction with low temperature partitioning followed by LC-MS/MS analysis. The method was validated for accuracy, precision, and sensitivity. The results show that the method is suitable for the determination of these antibiotics in animal products. The method was applied to the analysis of 2'600 samples, and the results showed a non-compliance rate of 0.81%.

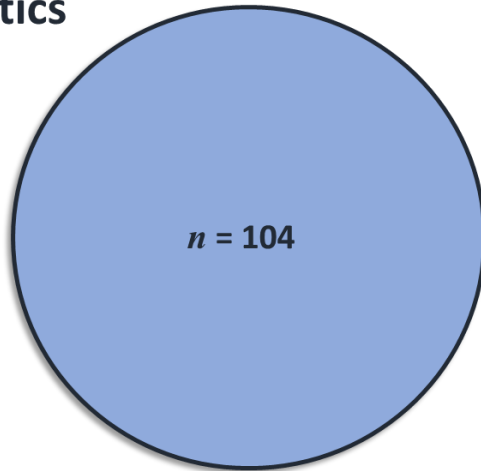
2018

Substances for screening

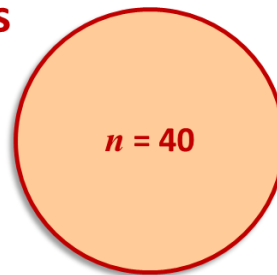
Antibiotics

- β-Lactams (23)**
- Sulfonamides (21)**
- Quinolones (18)**
- Aminoglycosides (14)**
- Tetracyclines (10)**
- Macrolides (7)
- Amphenicols (3)
- Diaminopyrimidines (2)
- Lincosamides (2)
- Rifamycins (2)
- Aminocoumarins (1)
- Streptogramins (1)

- Benzimidazoles (14)**
- Coccidiostats (11)**
- Avermectins (6)
- Salicylanilides (4)
- Halogenated phenols (1)
- Imidazothiazoles (1)
- Organophosphorous compounds (1)
- Praziquantel*
- Pyrantel*

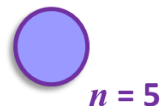


Antiparasitics



Anti-inflammatories

- Carprofen*
- Diclofenac*
- Flunixin*
- Meloxicam*
- Phenylbutazone*

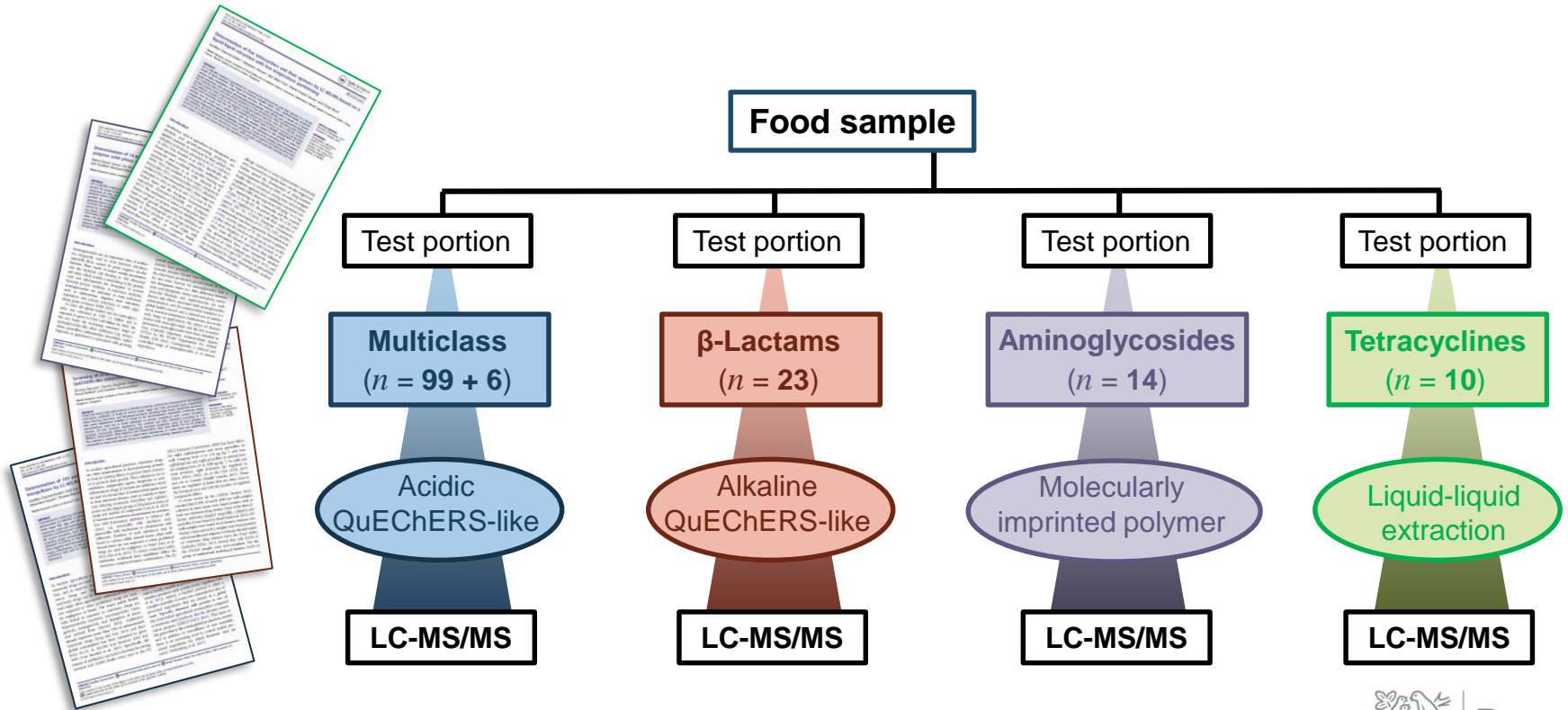


Tranquilizers

- Carazolol*
- Chlorpromazine*
- Xylazine*



A streamlined LC-MS/MS platform



A streamlined LC-MS/MS platform

& Background & Challenges

FOOD: ADJUTIVES & CONTAMINANTS: PART A, 2018
DOI: 10.1002/9781119440000.ch113.ch1333
<https://doi.org/10.1002/9781119440000.ch113.ch1333>

Taylor & Francis
Taylor & Francis Group

Check for updates

Screening of veterinary drug residues in food by LC-MS/MS. Background and challenges

Thierry Delatour, Lucie Racault, Thomas Bessaire and Aurélien Desmarchelier
Nestlé Research Center, Institute of Food Safety and Analytical Science, Lausanne, Switzerland

Food sample

Test portion

Multiclass
($n = 99 + 6$)

Acidic
QuEChERS-like

LC-MS/MS

Test portion

β -Lactams
($n = 23$)

Alkaline
QuEChERS-like

LC-MS/MS

Test portion

Aminoglycosides
($n = 14$)

Molecularly
imprinted polymer

LC-MS/MS

Test portion

Tetracyclines
($n = 10$)

Liquid-liquid
extraction

LC-MS/MS

Stability study

in standard solutions



Taylor & Francis
Taylor & Francis Group



Stability study of veterinary drugs in standard solutions for LC-MS/MS screening in food

Aurélien Desmarchelier*, Philipp Feuerriegel*, Hassan Fathi Ahmed*, Julie Moulin*, Andrea Beck*,
Claudia Mujahid*, Thomas Bessaire*, Marie-Claude Saroy* and Pascal Mottier*

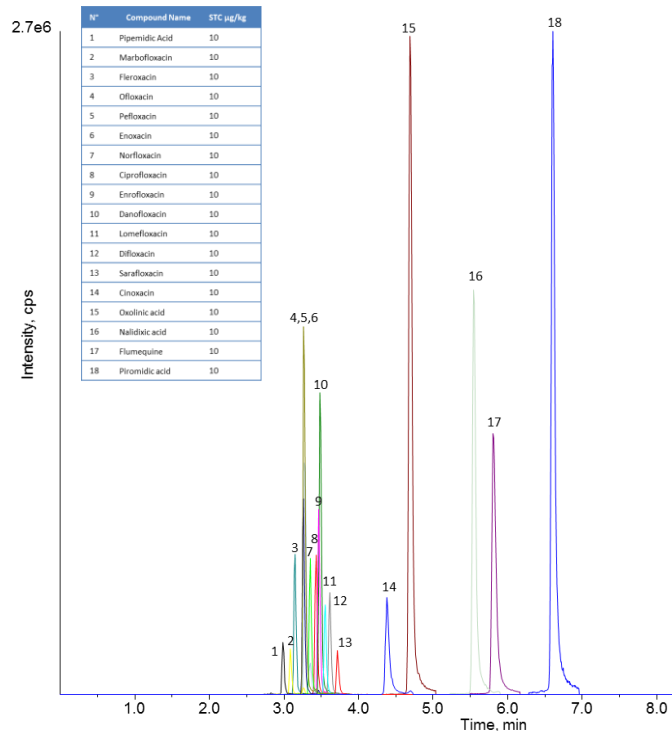
*Nestlé Research Center, Institute of Food Safety and Analytical Science, Lausanne, Switzerland; †CC Labor GmbH, Analytical Service,
Augsburg, Germany

FOOD ADJUTIVES & CONTAMINANTS: PART A, 2018
DOI: 10.1002/9781119440000.ch113.ch1333
<https://doi.org/10.1002/9781119440000.ch113.ch1333>

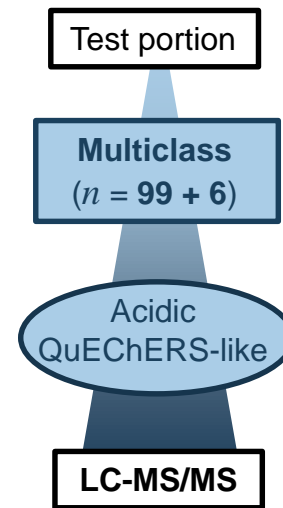
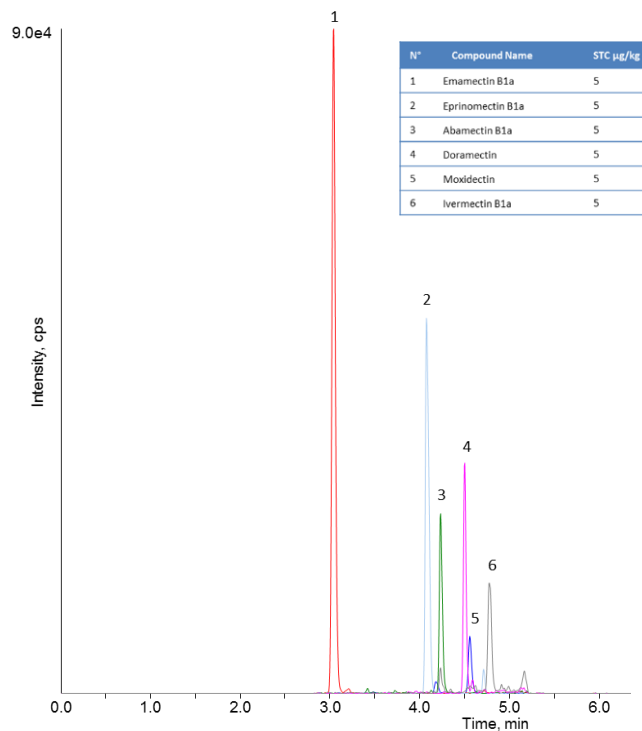
Stream 'Multiclass'

1xSTC (screening target concentration)

Quinolones in beef

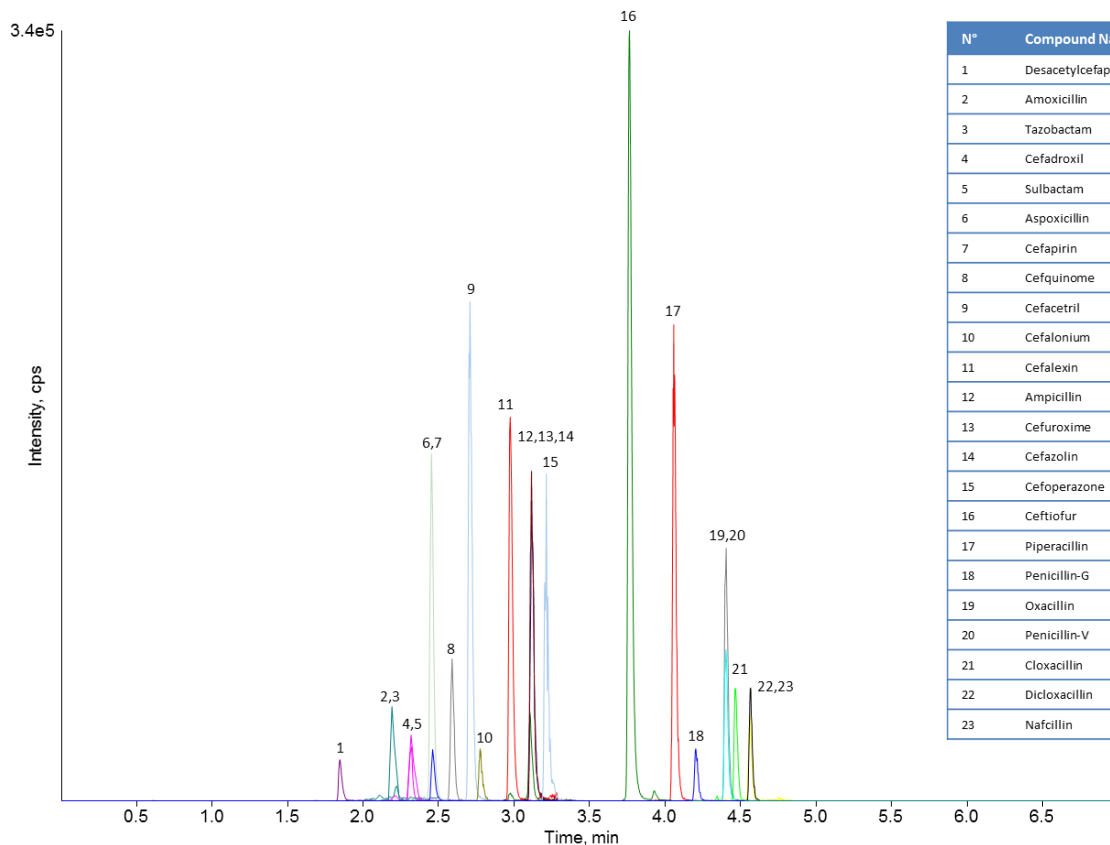


Avermectins in salmon

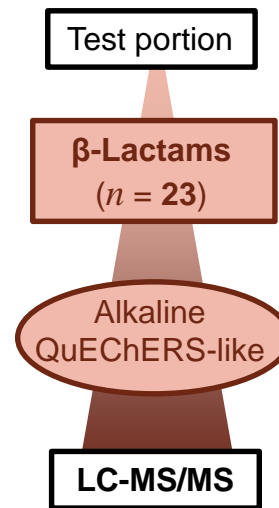


Stream 'β-Lactams'

Skimmed milk powder at 1xSTC



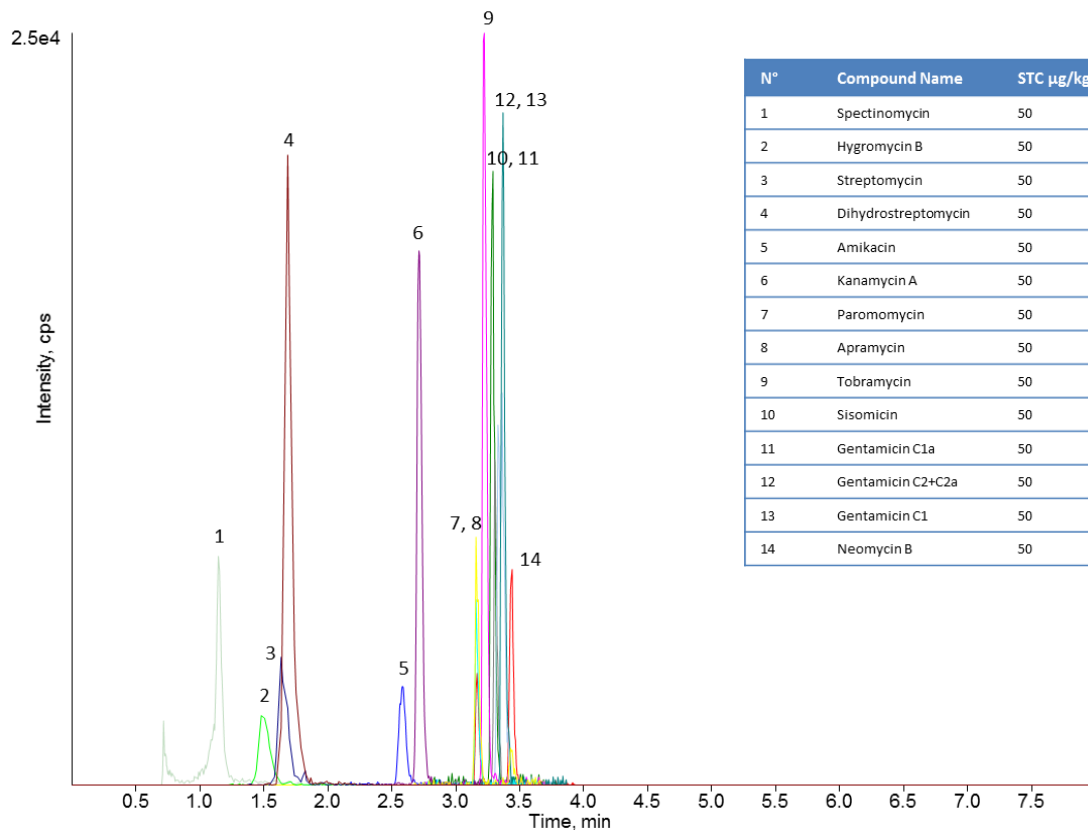
N°	Compound Name	STC µg/kg
1	Desacetylcefapirin	20
2	Amoxicillin	4
3	Tazobactam	25
4	Cefadroxil	50
5	Sulbactam	25
6	Aspoxicillin	25
7	Cefapirin	10
8	Cefquinome	10
9	Cefacetril	50
10	Cefalonium	10
11	Cefalexin	50
12	Ampicillin	4
13	Cefuroxime	50
14	Cefazolin	50
15	Cefoperazone	50
16	Ceftiofur	50
17	Piperacillin	10
18	Penicillin-G	4
19	Oxacillin	10
20	Penicillin-V	10
21	Cloxacillin	10
22	Dicloxacillin	10
23	Nafcillin	5



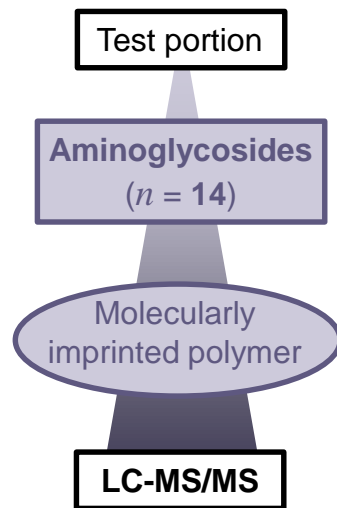
Research

Stream 'Aminoglycosides'

Egg powder at 1xSTC



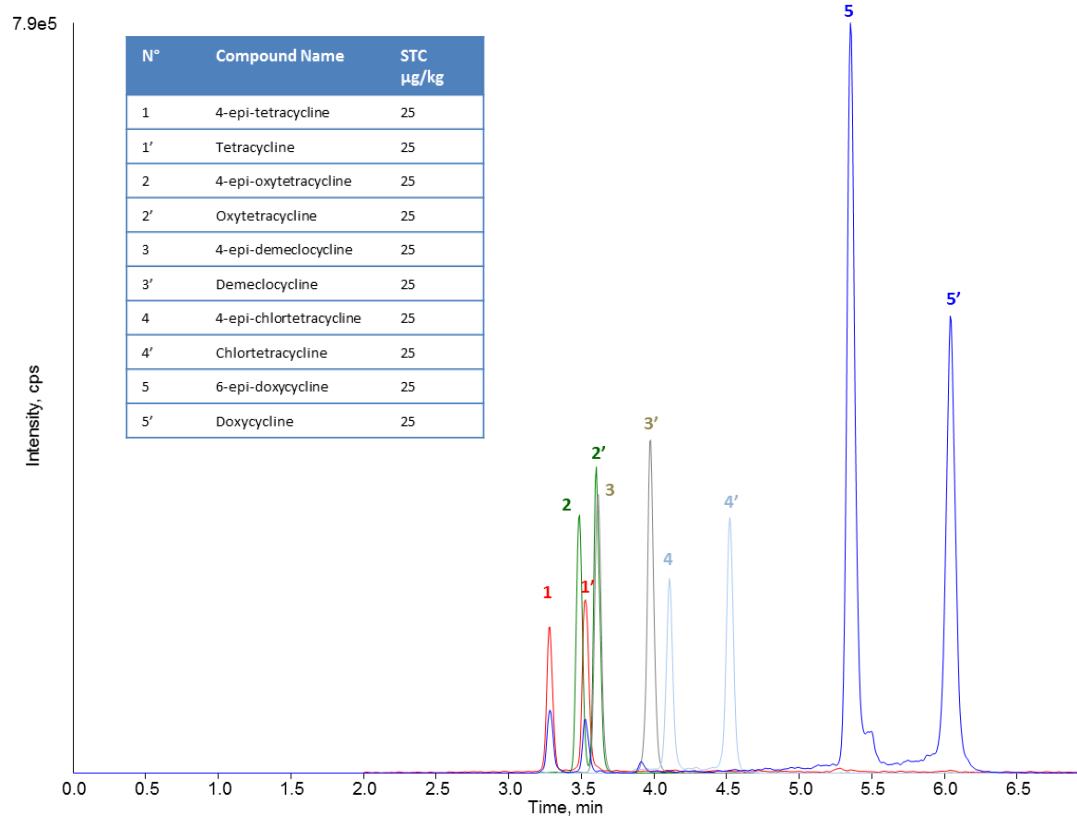
N°	Compound Name	STC µg/kg
1	Spectinomycin	50
2	Hygromycin B	50
3	Streptomycin	50
4	Dihydrostreptomycin	50
5	Amikacin	50
6	Kanamycin A	50
7	Paromomycin	50
8	Apramycin	50
9	Tobramycin	50
10	Sisomicin	50
11	Gentamicin C1a	50
12	Gentamicin C2+C2a	50
13	Gentamicin C1	50
14	Neomycin B	50



Research

Stream 'Tetracyclines'

Meat-based babyfood at 1xSTC



Test portion

Tetracyclines
($n = 10$)

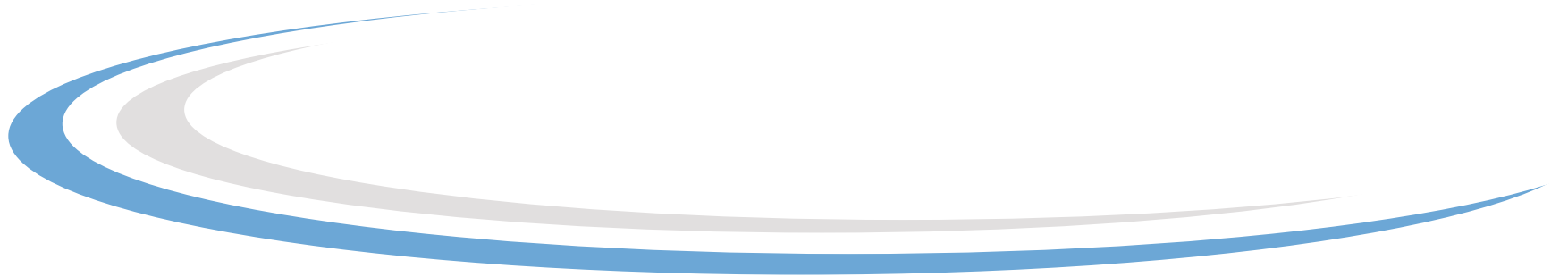
Liquid-liquid
extraction

LC-MS/MS



Research

What's screening about?



Research

What's screening about?

Absent *versus* **Present**

Low *versus* **High**

Compliant *versus* **Violative**

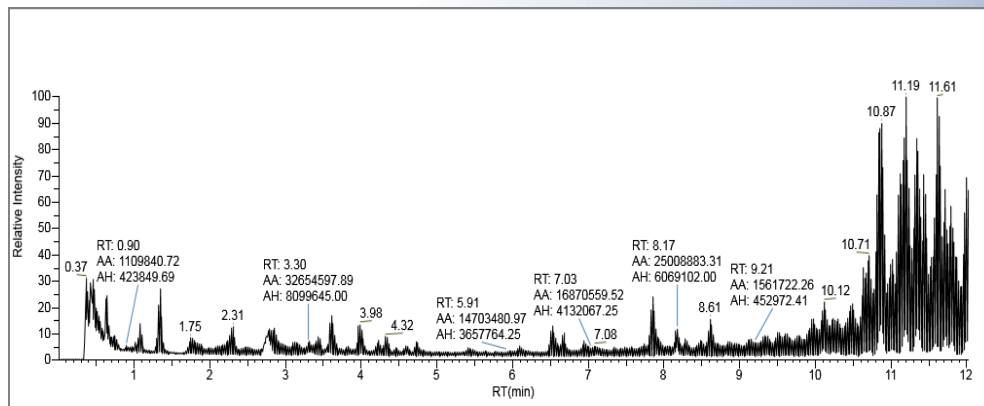
Free *versus* **Suspect**



Variation is a challenge for electrospray!

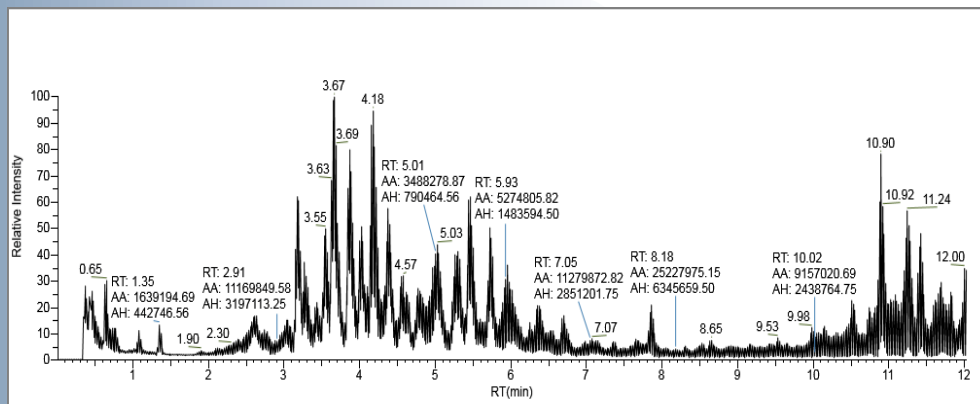


Variation of food composition



Pork powder

Hypoallergenic infant formula

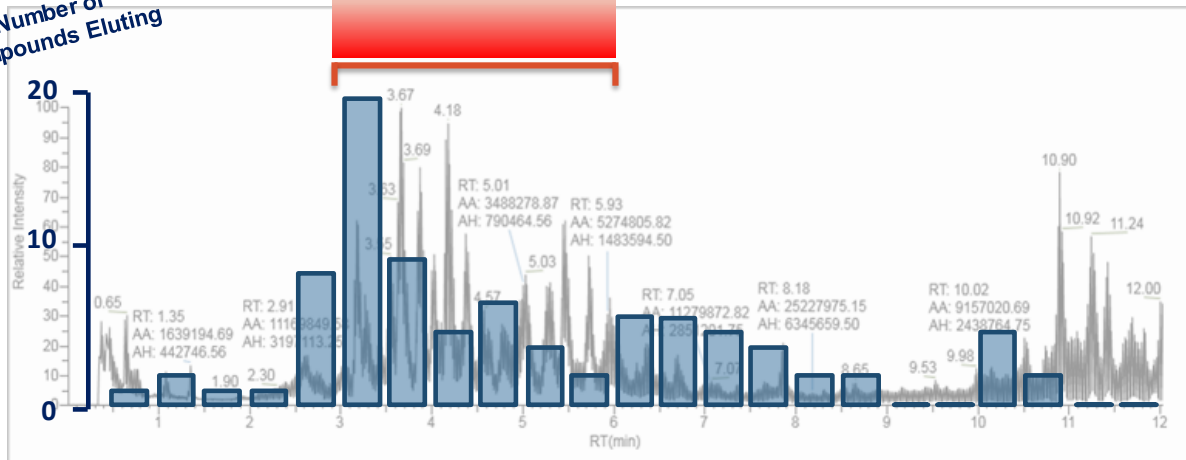


Research

Interference with the matrix

**56% of the analytes
elutes in the range 3-6 min**

Number of
Compounds Eluting



Hypoallergenic infant formula



Research

Process efficiency (PE)

Rec_{Abs} (%)

ME (%)

PE (%)

Absolute recovery

$$= \frac{\text{Area}_{\text{Sample}}}{\text{Area}_{\text{Extract}}}$$

Matrix effect

$$= \frac{\text{Area}_{\text{Extract}}}{\text{Area}_{\text{Standard}}}$$

Area_{Sample}

Signal of a sample spiked before extraction

Area_{Extract}

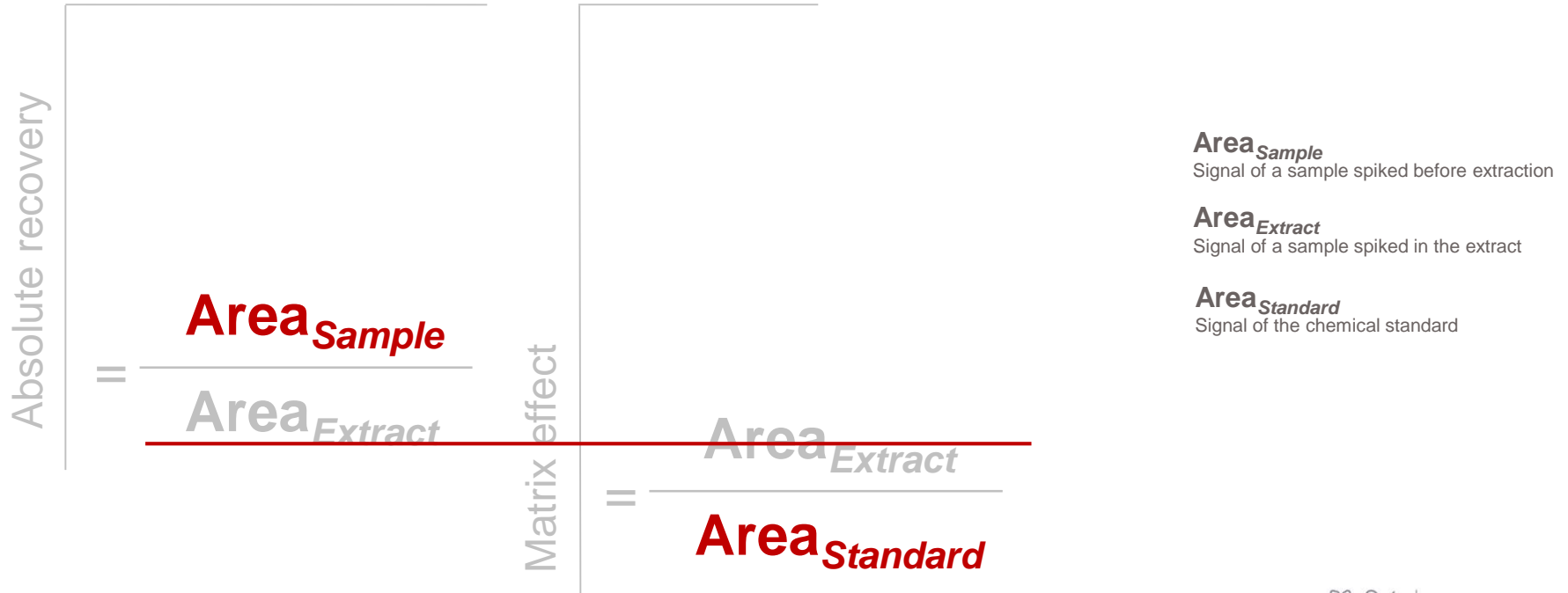
Signal of a sample spiked in the extract

Area_{Standard}

Signal of the chemical standard

Process efficiency (PE)

$$\text{Rec}_{Abs} (\%) \times \text{ME} (\%) = \text{PE} (\%)$$



Area_{Sample}
Signal of a sample spiked before extraction

Area_{Extract}
Signal of a sample spiked in the extract

Area_{Standard}
Signal of the chemical standard

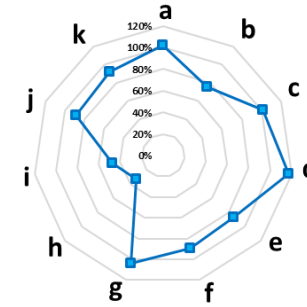
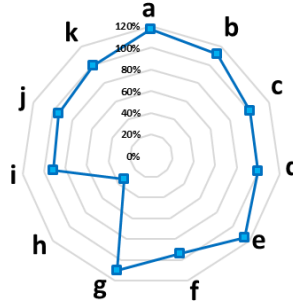
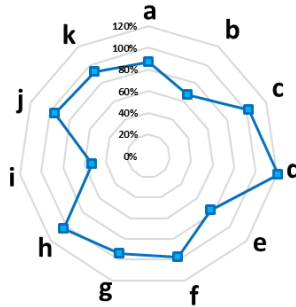
Process efficiency (PE)

Rec_{Abs} (%)

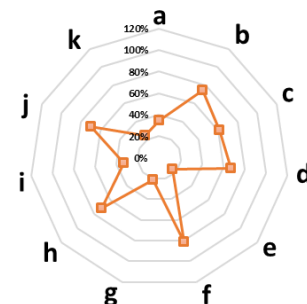
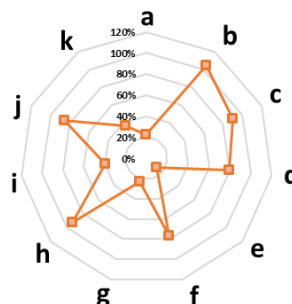
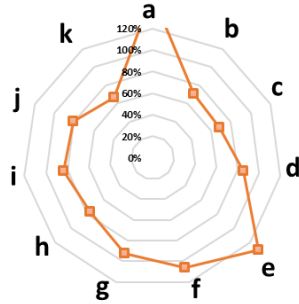
ME (%)

PE (%)

Rifaximin



Amprolium



Infant formulae: **a, b, c**

Milk powders: **d, e**

Baby food: **f**

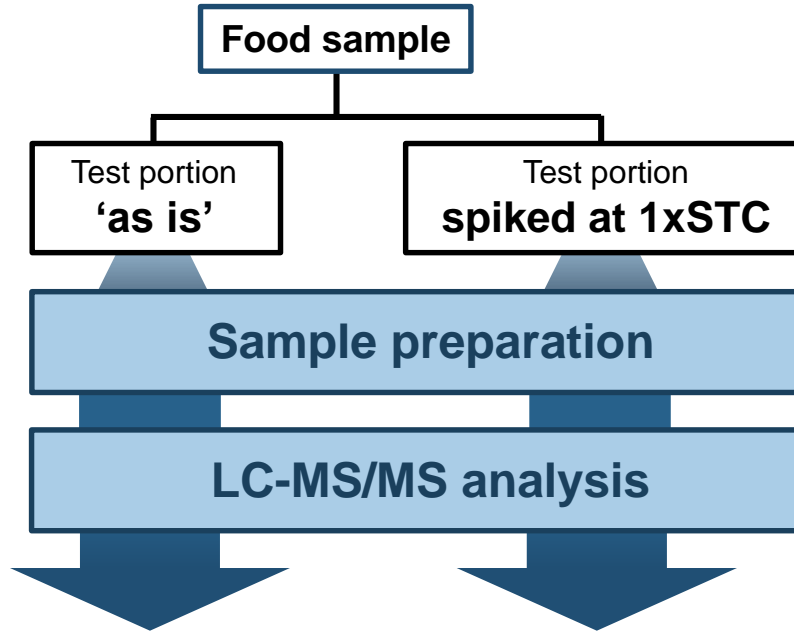
Meat powder: **g**

Fresh meat: **h, i**

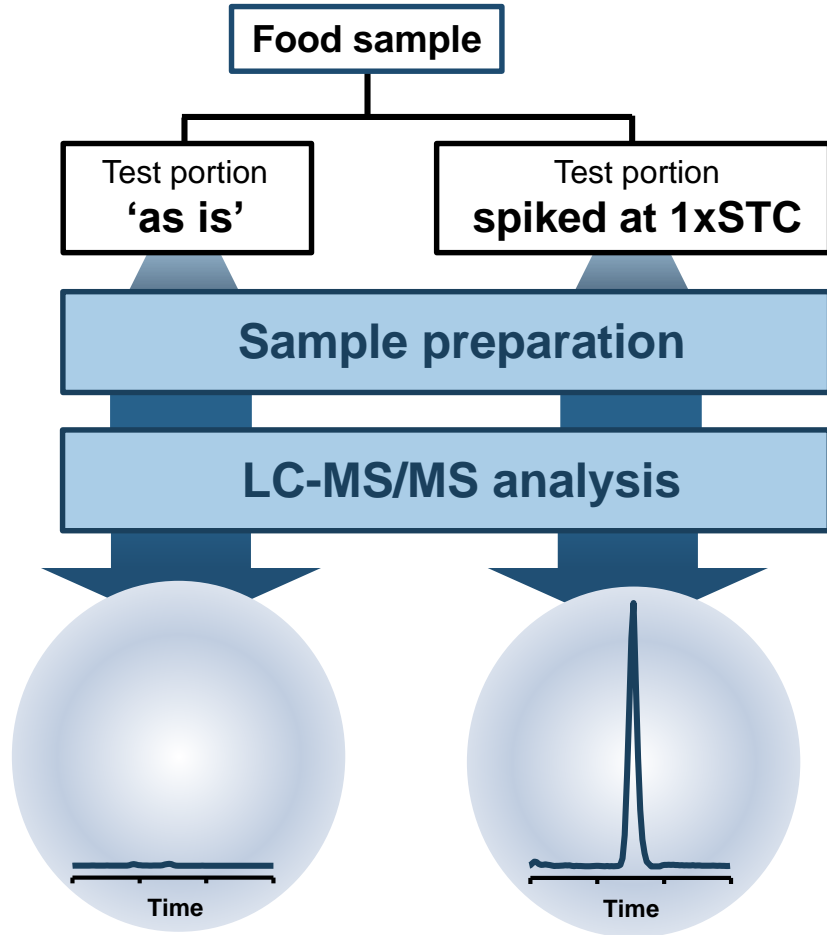
Fish: **j**

Infant cereal: **k**

Sample workflow



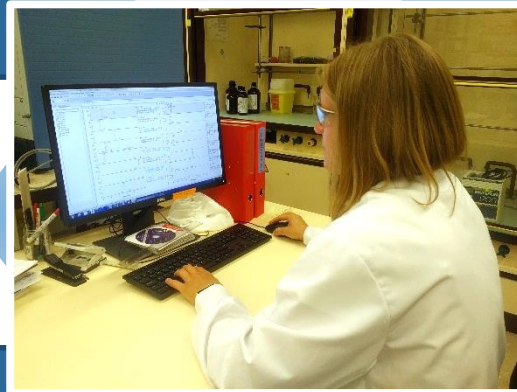
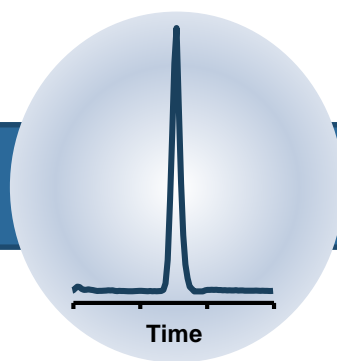
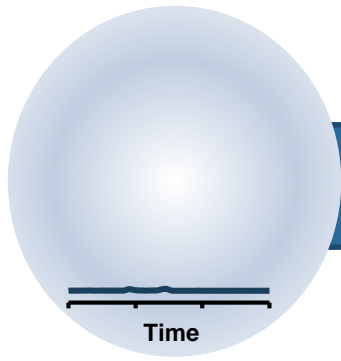
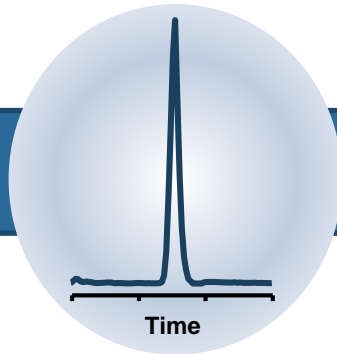
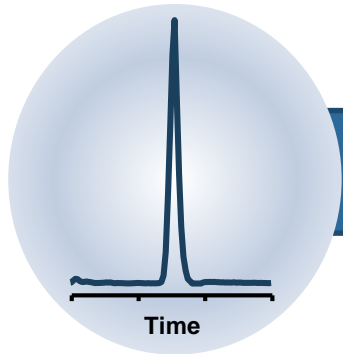
Sample workflow



Effective & reliable review of the data

'As is'

Spiked at 1xSTC

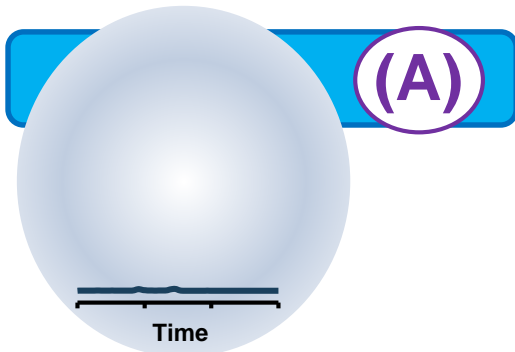
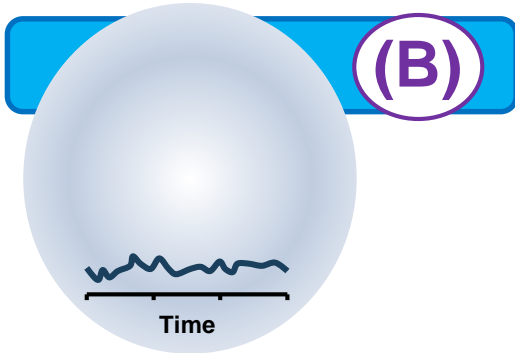


Rejected

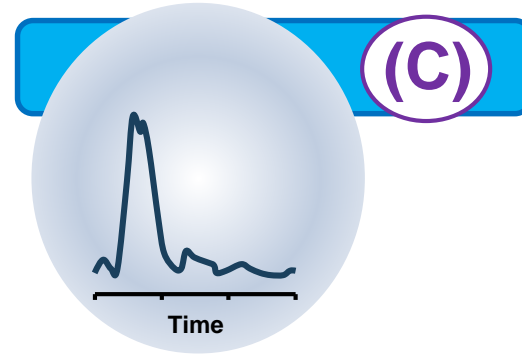
Approved

Effective & reliable review of the data

'As is'



'As is'



(A): Sample is negative

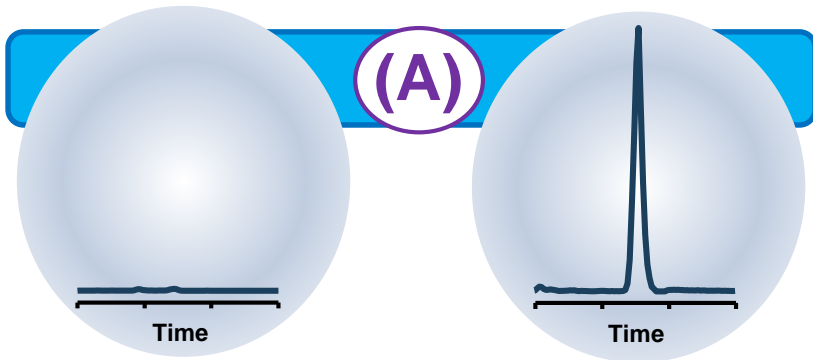
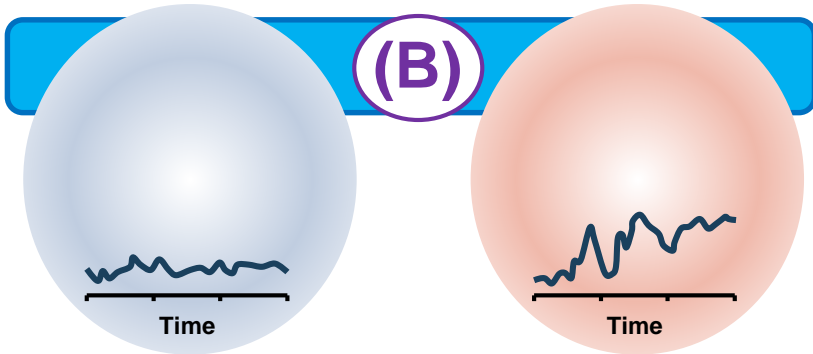
(B): Sample is negative

(C): Sample is positive

Effective & reliable review of the data

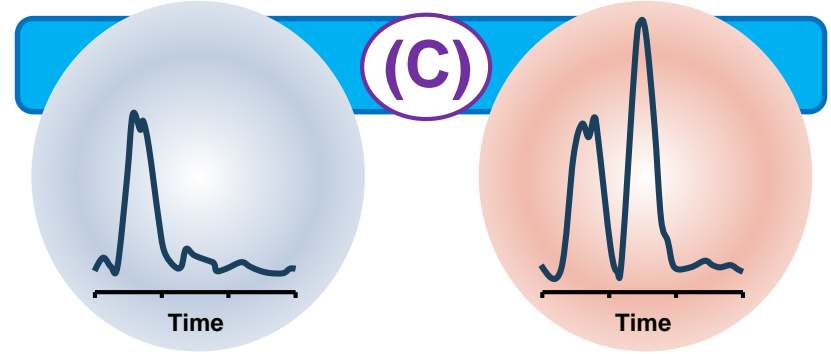
'As is'

Spiked at 1xSTC



'As is'

Spiked at 1xSTC

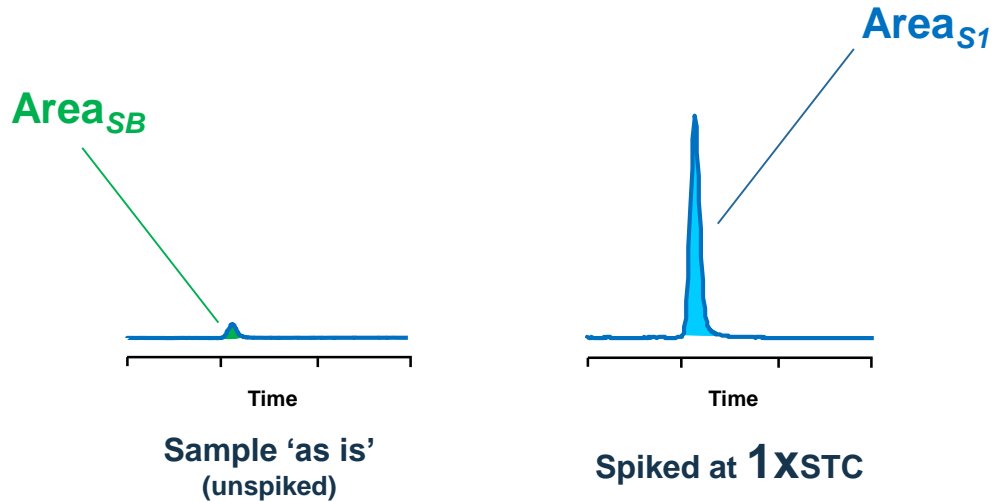


(A): Sample is negative

(B): Sample **has an odd behavior**

(C): Sample **is negative**

Screening based on absolute areas



Screening based on area ratios

$Area_{SB}$



Time

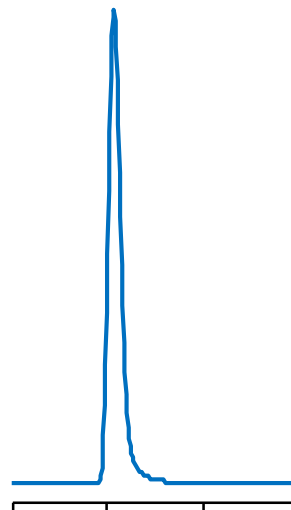
Sample 'as is'
(unspiked)

$Area_{S1}$



Time

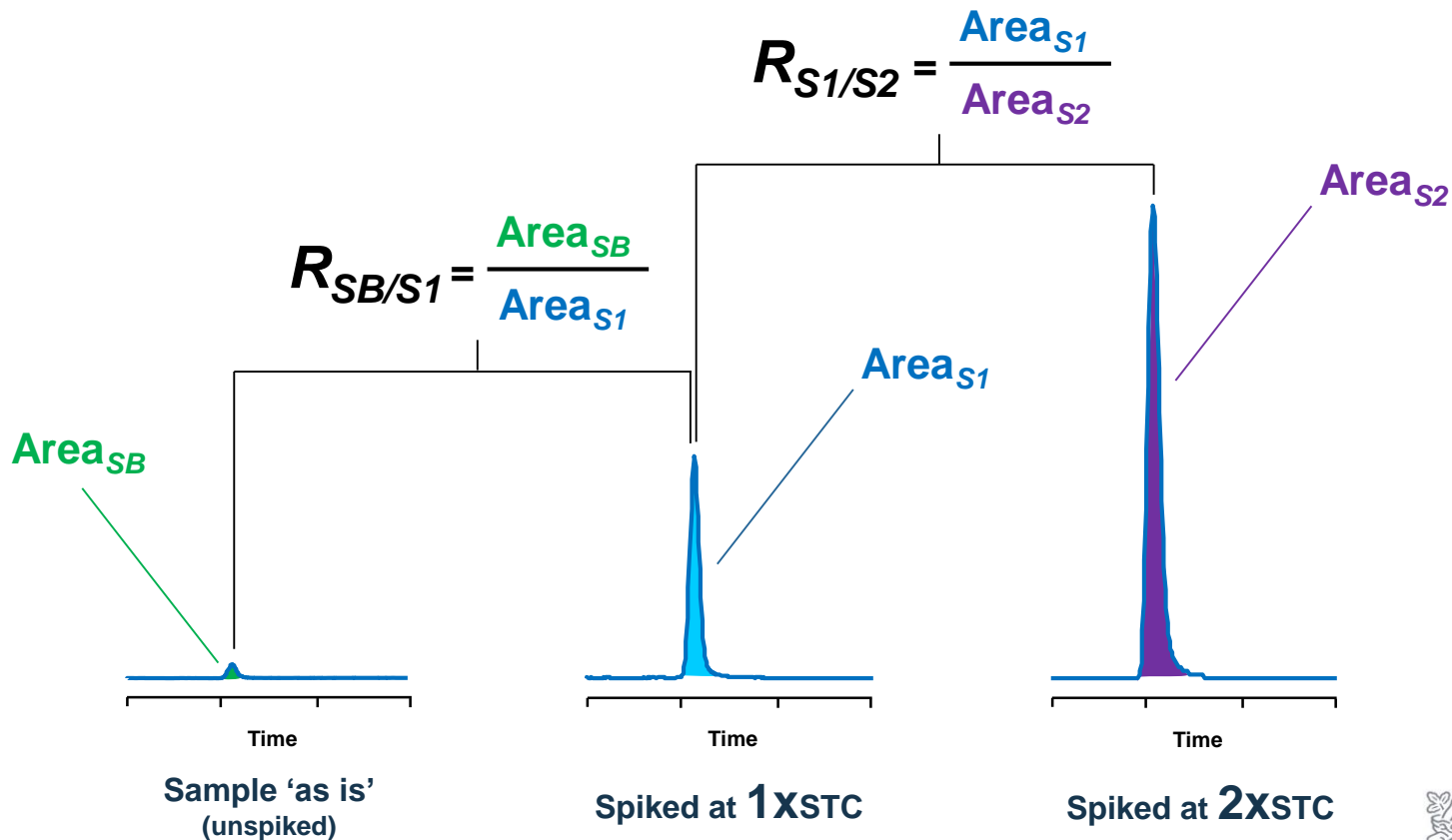
Spiked at **1XSTC**



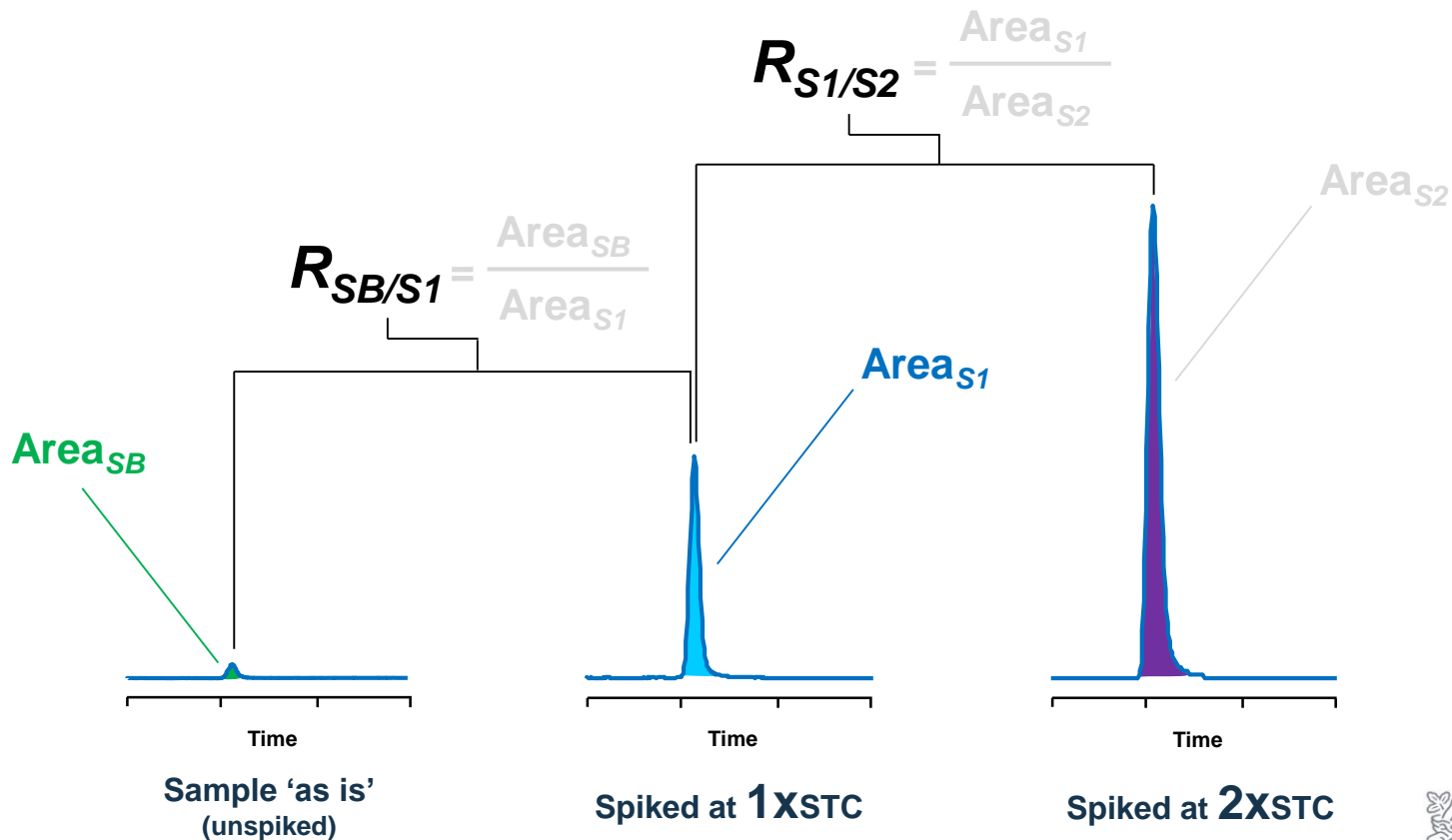
Time

Spiked at **2XSTC**

Screening based on area ratios



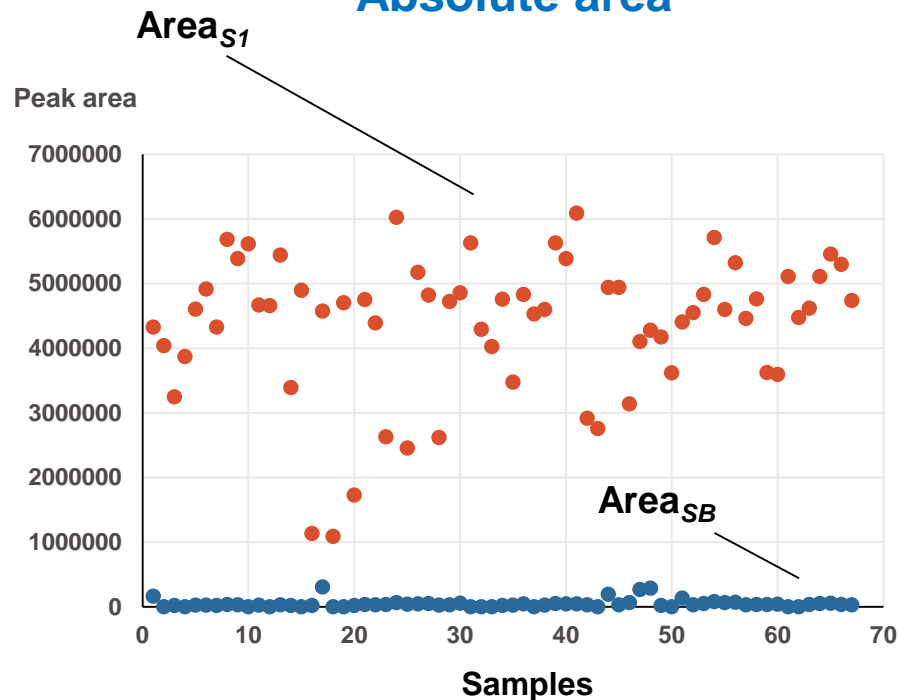
Assessment of screening performance



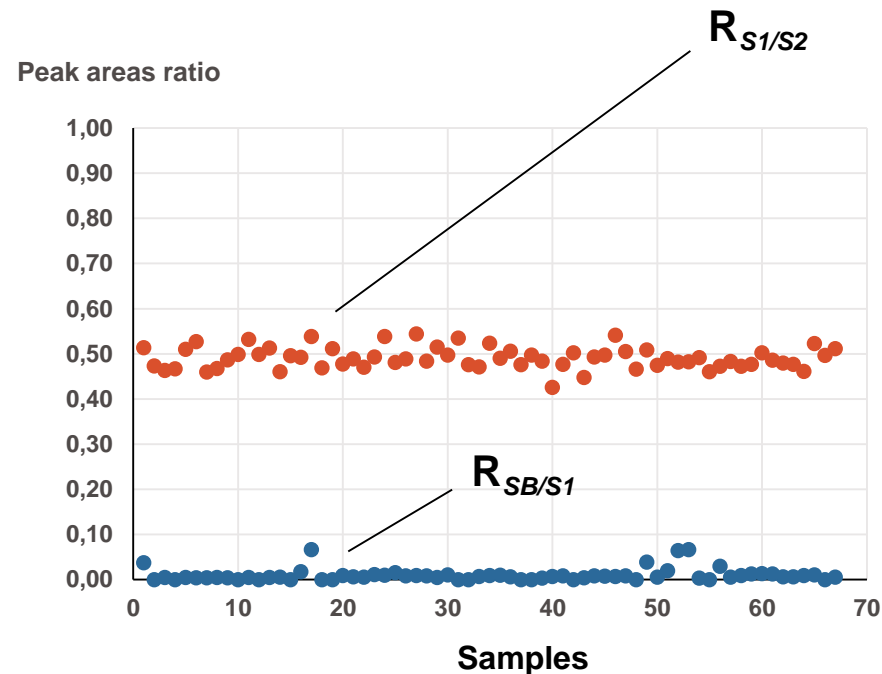
Signal response in food samples

5-Hydroxythiabendazole

Absolute area



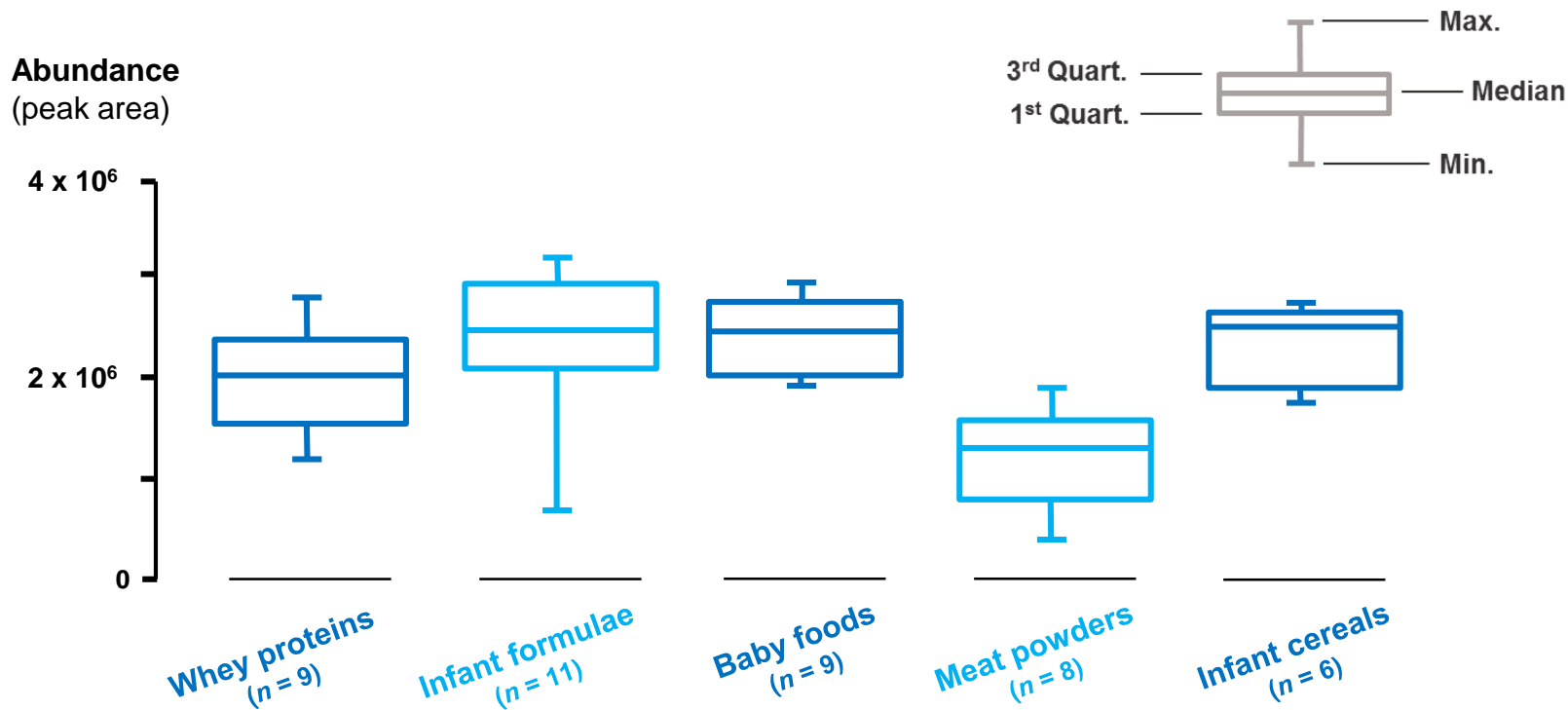
Area ratio



● Sample at 1xSTC

● Blank sample

Response per category – Screening with absolute area

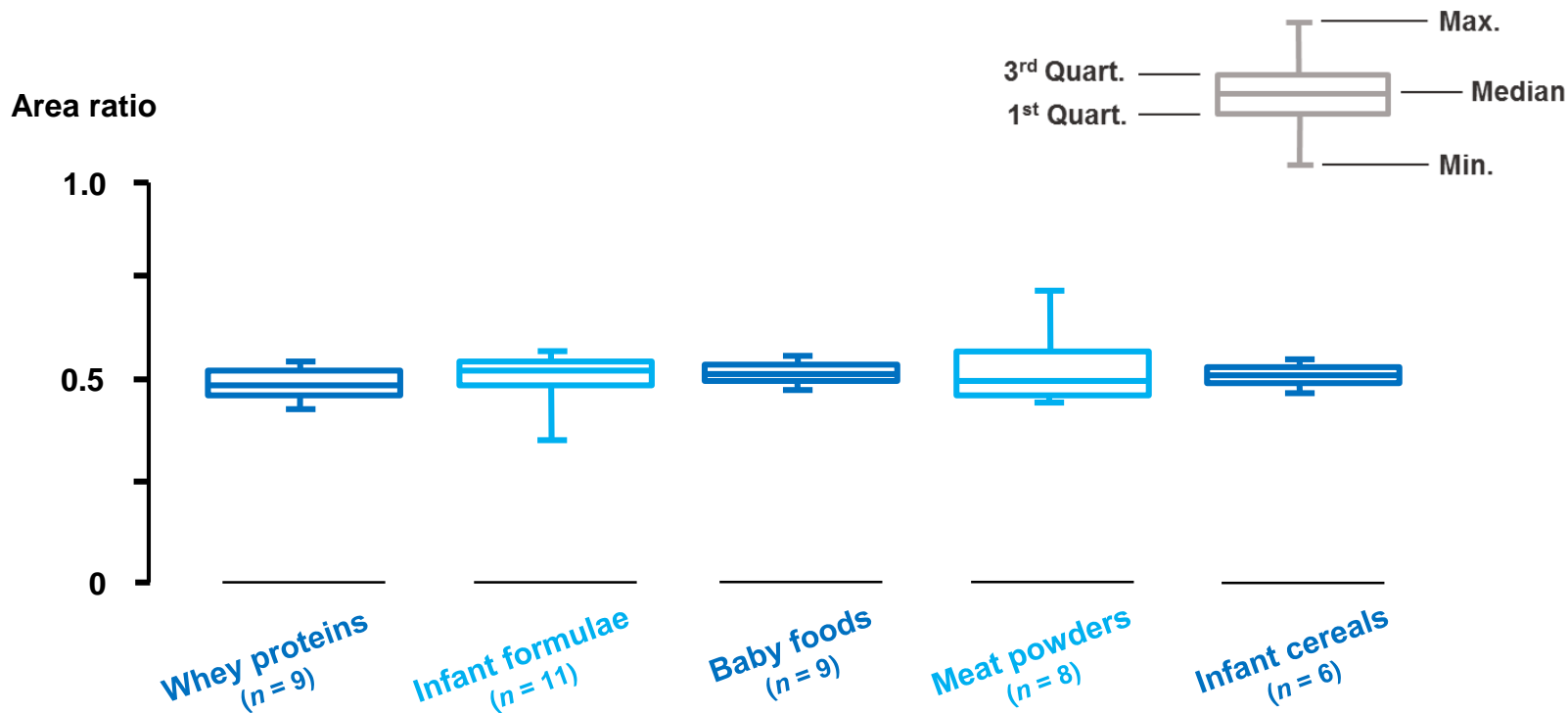


5-Hydroxythiabendazole at 1xSTC



Research

Response per category – Screening with area ratio



5-Hydroxythiabendazole at 1xSTC



Research

From variation within a category ...



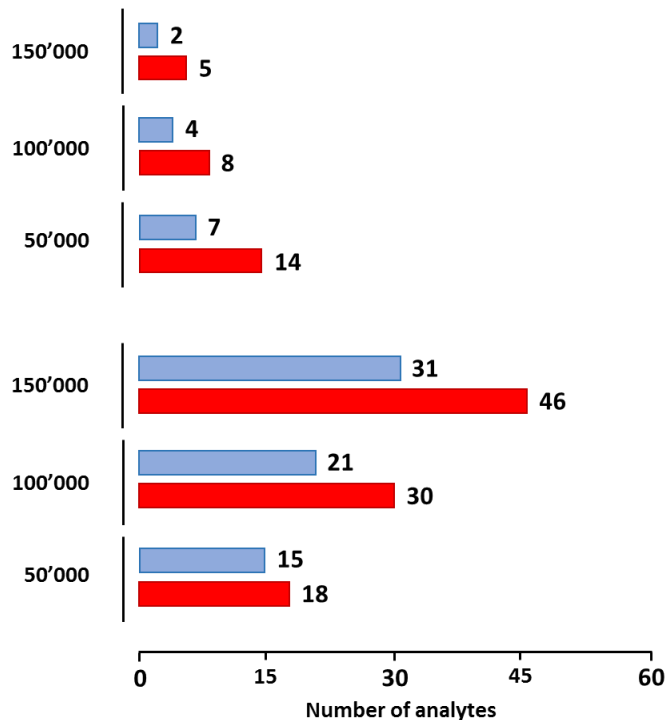
... to variation across categories



False response rates ($n = 105$ analytes)

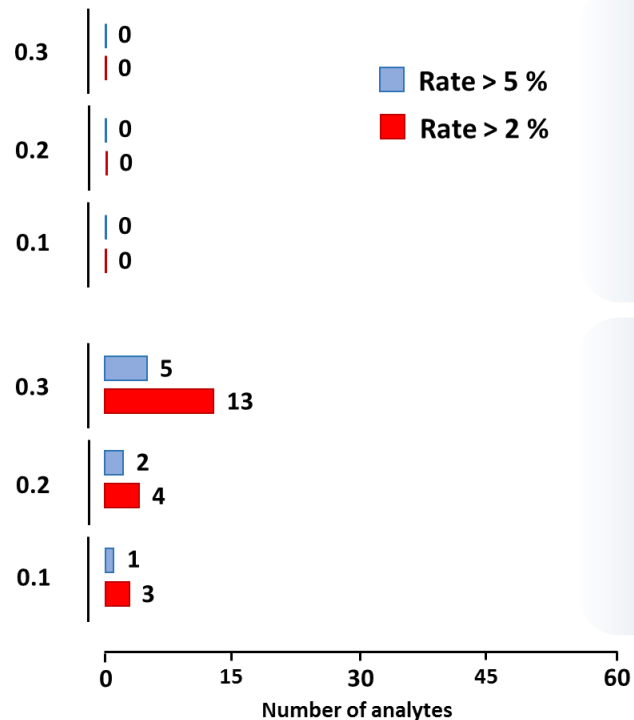
Absolute area

Threshold value



Area ratio

$V_{\text{cut-off}}$



False positives

False negatives

Take home message

- ▶ **Liquid chromatography-mass spectrometry is a suitable technique for multiresidue screening of more than a hundred veterinary drug residues in food.**
- ▶ **Instability of electrospray mediated by food composition variation is a limitation for screening in a broad range of commodities.**



- ▶ **The 'Area ratio' approach improves the efficiency and the reliability of the data review.**
- ▶ **The 'Area ratio' approach significantly improves the screening performance in terms of false responses.**



Thanks for your attention



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Research